The bone-protective effect of a Taiwanese yam (Dioscorea alata L. cv. Tainung No. 2) in ovariectomised female BALB/C mice

Hsiao-ling Chen,a∗† Ling-tiao Hong,a Jong Kang Leeb and Ching-jang Huangc†

Abstract

BACKGROUND: Yam products have been marketed for treating postmenopausal syndromes. This study investigated the effects of Dioscorea alata L. cv. Tainung No. 2 (TNG yam) on the bone density of ovariectomised (OVX) female BALB/c mice and the mechanism whereby TNG yam exerted this effect. Sham and OVX control groups were fed a control diet while remaining OVX mice were randomly allocated into experimental diets, i.e. yam (630 g TNG powder kg−1), E2 (20 mg 17β-oestradiol kg−1), or genistein (2 g genistein kg−1) diet. After 12 weeks of feeding, the uterine weight, indices of bone mass and caecal short chain fatty acids were determined.

RESULTS: Neither a yam nor genistein diet restored the OVX-induced uterine atrophy as did the E2 diet. The femoral and lumbar bone mineral density (BMD) of mice fed the yam diet was greater than those of the sham group, respectively (P < 0.05 vs OVX control), while the lumbar BMD of yam and sham groups were similar (P > 0.05 vs sham). The femoral ash and calcium content in the yam group was significantly greater than that in the OVX control group, respectively (P < 0.05 vs OVX control). The total short chain fatty acid content in the caecum, only enhanced in the yam group, was not correlated with the calcium content of either bone or the plasma calcium level.

CONCLUSION: TNG yam prevented loss of BMD and improved bone calcium status without stimulating uterine hypertrophy in OVX BALB/c mice. TNG yam may be beneficial for postmenopausal women for preventing bone loss.

Keywords: ovariectomised; Dioscorea alata; yam; genistein; bone mineral density; calcium; short chain fatty acid

INTRODUCTION

Yam is the common name of rhizomes of a perennial plant from the genus Dioscorea in the family Dioscoreaceae. It is estimated that there are more than 600 species of yams in the world, 14 of which are found in Taiwan.1 There are five major cultivated species of Taiwanese yam: D. alata, D. batatas, D. japonica, D. alata L. var purpurea, and D. doryophora. Among these, D. alata cv. Tainung No. 2 (TNG) is one of the most consumed varieties.1

Most of the worldwide production of yam is located in Africa where yam has been traditionally used as a staple food. In addition, yam has been widely used for the enhancement of health in oriental countries and traditionally considered as a superior Chinese herb to improve gastrointestinal function.2 Recently, Wang et al.3 have demonstrated that yam-rich diets enhance caecal fermentation and proliferation of bifidobacteria. Yams have also been implicated in the promotion of the health of postmenopausal women. The wild Mexican yam has been marketed for treating postmenopausal syndromes. Wu et al.4 demonstrate that replacing two-thirds of staple food with Taiwanese yam (D. alata) in postmenopausal women for 30 days improves the status of sex hormones, which suggests that D. alata may contain phytoestrogenic compounds. Recently, Cheng et al.5 identified five phytoestrogenic compounds, γ-tocopherol-9, coenzyme Q9, 1-feruloylglycerol, α-tocopherol and hydro-Q9 chromene, in TNG yam based on the ligand-dependent transactivation assay of oestrogen receptors, which further supports the phytoestrogenic potential of TNG yams.

Ovarian hormone deficiency is a major risk factor for osteoporosis in postmenopausal women.6 Hormone replacement therapy (HRT) effectively alleviates postmenopausal symptoms and lowers the risk for coronary heart disease and osteoporosis. However, a longitudinal study indicates more than 80% of 45–55-year-old women are unwilling to be placed on this therapy, either be-
cause of the concern of increased risk of certain types of cancer or contraindications.1,2 Phytoestrogens, plant-derived compounds that can interact with oestrogen receptors and exhibit oestrogenic activities,3,4 have been considered an alternative remedy for HRT. Soy isoflavones, a group of well-known phytoestrogens, have been shown to exert protective potential against bone loss in several animal and tissue studies.3,10 Epidemiological studies also generally suggest a positive association between soy consumption and bone mineral density.1,11 In addition, resistant starch has been shown to enhance calcium absorption probably by enhancing colonic fermentation.12,13 TNG yam, sources of potential phytoestrogens and resistant starch,3 may protect against bone loss in postmenopausal women both through the direct phytoestrogenic action on tissues and the enhancing effect on calcium absorption.

To examine the bone-protective potency of TNG yam, we fed ovariectomised (OVX) BALB/c mice with a yam diet for 12 weeks, and compared the effect of yam with those of 17β-oestradiol (E2) and genistein, a soy phytoestrogen, on bone mineral density (BMD), ash and calcium contents, as well as serum calcium level. Colonic short chain fatty acids were determined to explore whether the fermentation of yam contributed to the bone-protective role of yam.

MATERIALS AND METHODS
Preparation and analysis of yam powder
Dioscorea alata L. cv. Tainung No. 2 (TNG) was purchased from its major production area (Farmer’s Association, Nantao County, Taiwan). The whole yam tubers were peeled, steamed, lyophilised, and milled to powder. The protein, fat, ash, calcium, magnesium and phosphorus content of the lyophilised yam powder and corn starch were analysed according to the standards of the Association of Official Analytical Chemists (AOAC) methods.14 Protein level was determined by Kjeldahl analysis for nitrogen concentration.14 Fat was extracted by using ether in a Soxhlet apparatus.14 Ash was determined by heating at 550 °C overnight.14 Moisture level was determined by vacuum drying at 110 °C overnight.14 The mineral content was determined using an atomic absorption spectrometer (PE3300; Perkin–Elmer, Norwalk, CT, USA). Dietary fibre contents were analysed using enzymatic–gravimetric method.15 Resistant starch was analysed according to the AOAC method with a commercial kit (RSTAR11/02; Megazyme, Bray, Ireland).15 The carbohydrate level was calculated by subtracting protein, fat, ash, dietary fibre and moisture levels (Table 1).

Experimental design
Female BALB/c mice (4 weeks old) were obtained from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). They were housed individually in solid-bottomed plastic cages with wood shavings for bedding, in a room maintained on a 12-h light/12-h dark cycle (0800–2000), at 24 ± 1 °C and 50% humidity. All animals were allowed free access to water and food during the study. Animal care followed the guidelines of the National Research Council16 and was approved by the Institutional Animal Care and Use Committee in the Chung Shan Medical University. Mice were initially fed a standard non-purified diet (Rodent Laboratory Chow; Purina Mills, St Louis, MO, USA) until they were undergone sham or OVX surgery at 8 weeks of age. Three days later, OVX mice were assigned randomly to consume control (modified corn oil AIN-93 diet), yam (630 g TNG powder kg⁻¹ diet), oestradiol-supplemented (2 mg 17β-oestradiol kg⁻¹ diet; Sigma, St Louis, MO, USA), or genistein-supplemented (0.2 g genistein kg⁻¹ diet; Sigma) diets for 12 weeks, while the sham group was fed the control diet. The dose of genistein used in the study followed the study by Ward et al.17 while the dose of 17β-oestradiol in the diet was based on the relative binding affinity of genistein and 17β-oestradiol to the oestrogen receptor-α.2 The composition (in g kg⁻¹) of control diet was corn starch, 629.5; corn oil, 70; casein, 200; cellulose, 50; mineral mix, 35; vitamin 10; γ -l-cystine, 3; and choline bitratear, 2.5. All diets contained corn oil instead of soy oil, to eliminate any food intake was monitored every day. Mice were decapitated after 12 weeks of study without fasting. Blood was collected into heparinised tubes, while both the femur and the lumbar bones (L3–5) were dissected for further analysis. Caecal contents were removed, weighed, and stored at −80 °C for further analyses of short chain fatty acid (SCFA).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Yam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient content (g kg⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>549.9</td>
<td>360.3</td>
</tr>
<tr>
<td>Resistant starch</td>
<td>0.0</td>
<td>94.6</td>
</tr>
<tr>
<td>Protein</td>
<td>200.0</td>
<td>291.8</td>
</tr>
<tr>
<td>Fat</td>
<td>70.0</td>
<td>72.9</td>
</tr>
<tr>
<td>Dietary fibre</td>
<td>50.0</td>
<td>83.4</td>
</tr>
<tr>
<td>Ca</td>
<td>5.2</td>
<td>5.1</td>
</tr>
<tr>
<td>P</td>
<td>2.0</td>
<td>3.5</td>
</tr>
<tr>
<td>M</td>
<td>0.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Energy density (kcal g⁻¹)</td>
<td>3.6</td>
<td>3.3</td>
</tr>
<tr>
<td>Energy (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>60.6</td>
<td>44.2</td>
</tr>
<tr>
<td>Protein</td>
<td>22.0</td>
<td>35.8</td>
</tr>
<tr>
<td>Fat</td>
<td>17.4</td>
<td>20.1</td>
</tr>
</tbody>
</table>

Table 2. Nutrient content and nutritional values of the control and yam diets

Table 1. Composition of the corn starch and lyophilised cooked yam powder prepared from skinned yam tubera

<table>
<thead>
<tr>
<th>Major component (g kg⁻¹)</th>
<th>Corn starch</th>
<th>Yam powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>874.5 ± 0.6</td>
<td>722.7 ± 0.6</td>
</tr>
<tr>
<td>Resistant starch</td>
<td>0</td>
<td>150.8 ± 22.3</td>
</tr>
<tr>
<td>Protein</td>
<td>0</td>
<td>145.8 ± 0.5</td>
</tr>
<tr>
<td>Fat</td>
<td>0</td>
<td>0.46 ± 0.1</td>
</tr>
<tr>
<td>Ash</td>
<td>0.4 ± 0.1</td>
<td>40.4 ± 0.1</td>
</tr>
<tr>
<td>Total dietary fibre</td>
<td>0</td>
<td>5.30 ± 0.02</td>
</tr>
<tr>
<td>Moisture</td>
<td>125.1 ± 0.5</td>
<td>68.9 ± 3.0</td>
</tr>
<tr>
<td>Ca (10⁻³)</td>
<td>297.0 ± 4.0</td>
<td>132.4 ± 4.0</td>
</tr>
<tr>
<td>P (10⁻³)</td>
<td>68.2 ± 6.0</td>
<td>2398.0 ± 12.0</td>
</tr>
<tr>
<td>Mg (10⁻³)</td>
<td>60.8 ± 3.0</td>
<td>557.2 ± 31.0</td>
</tr>
</tbody>
</table>

a Data are means ± SD of three replicates.
Phytoestrogenic effects of a Taiwanese yam on bones www.soci.org

Radiographic analysis of the femur and lumbar bones
The bone densities (g cm\(^{-2}\)) of the femur and lumbar bones were measured by a dual-energy X-ray absorptiometry (Lunar Expert-XL, version 1.8; Lunar-Norland Medical Systems, Madison,WI, USA) in triplicate. The speed of scan was 3 cm s\(^{-1}\); with a resolution of 0.6 mm. The coefficient of variation for the femur and lumbar bone was 2.5% and 5%, respectively.

Histology of femoral cross-sections
In a pilot study, we found OVX caused bone loss mainly in the region close to distal metaphysis of the femur. Therefore, a cross-section of the femur was prepared at this region. After being decalcified in ethylene diaminetetraacetic acid (EDTA, 30 g L\(^{-1}\)) phosphate buffer (0.1 mol L\(^{-1}\), pH 7.4) overnight, the right femur was embedded in tissue freezing medium (Leica Microsystems Nusslich GmbH, Wetzlar, Germany) for cryosection (Leica CM3050S; Bartels and Stout Inc., Issaquah, WA, USA). The section (5 mm) was stained with haemotoxylin (Merck, Darmstadt, Germany) and observed under a light microscope (E600; Nikon Corp., Tokyo, Japan). The thickness of the cortical bone and the trabecular bone fraction (trabecular bone volume in a cube/total volume of a cube\(^{16}\)) was quantified using Image Analysis Systems, (Lucia Measurement version 4.81; Laboratory Imaging Ltd., Praha, Czech Republic).

Bone ash and calcium contents
The ash contents of the femur and lumbar bones were measured according to the AOAC method.\(^{14}\) The ash was weighed and then diluted with nitric acid (atomic absorption spectrometer grade). The calcium concentration was determined with an atomic absorption spectrometer (PE3300; Perkin–Elmer).

Plasma calcium concentration
Plasma calcium was measured with a commercial kit (CA590-592; Randox, Antrim, UK) according to the protocol provided by the manufacturer. The plasma calcium concentration was calculated by comparison with appropriate standards.

Caecal short chain fatty acids
The caecal content was analysed for acetate, propionate and n-butyrate with 4-methyl-n-valeric acid as an internal standard, as described previously.\(^{19,20}\) SCFAs extracted from caecal contents were dissolved in a phosphate solution (100 g L\(^{-1}\)) just before they were injected onto a gas chromatography (GC-14B; Shimadzu, Tokyo, Japan) fitted with a glass capillary column (0.25 mm \(\times\) 30 m, Stabilwax-DA; Restek Corp., Bellefonte, PA, USA) and a flame ionisation detector. The initial oven temperature was 100 °C and was raised to 200 °C at 6 °C min\(^{-1}\). The temperature of the injection port and detector was 250 °C, respectively. The flow rate of carrier gas, N\(_2\), was adjusted to be 1 mL min\(^{-1}\). Peak areas were analysed with C-R6A Chromatopac (Shimadzu Corp.). Caecal SCFA content (mmol g\(^{-1}\) caecal content mass (g) at the time of sacrifice.

Statistical analysis
The Statistical Package for Social Science (SPSS for Windows, version 8.0; SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Values are presented as means with standard deviation. One-way ANOVA tests were used to determine the significance of differences among groups. The group-wise comparison was performed by Duncan's multiple range test.\(^{21}\) The correlation between caecal SCFA content (mmol caecum\(^{-1}\)) and femur, lumbar calcium content (mg), blood calcium level was determined by Pearson's correlation test.\(^{21}\) Effects were considered significant at \(P < 0.05\).

RESULTS

Body weight and feed intake
Compared to the sham-operated group, the OVX control mice did not differ in weight gain, feed intake and feed efficiency (\(P > 0.05\), Table 3). Supplementation with oestradiol tended to reduce the weight gain and food intake as compared to the OVX control. Despite that, the yam group consumed the greatest amount of diet as compared to all other OVX groups (\(P < 0.05\), the feed efficiency was similar among all five groups (\(P < 0.05\), Table 3). The daily calcium intake of sham, control, yam, oestriadiol and genistein groups was 18.18 ± 1.08, 17.27 ± 1.14, 19.16 ± 1.27, 16.60 ± 1.30 and 17.01 ± 1.40 mg, respectively, after the daily feed intake was taken into account.

Uterus weight
The uterus weight was significantly lower in the OVX control group, 22.4 ± 15.1 mg, than in the sham group, 102.1 ± 52.6 mg (\(P < 0.05\)). Supplementation with oestradiol effectively prevented the uterine atrophy of OVX; the uterus weight of the E2 group (139.5 ± 38.4 mg) (\(P < 0.05\) vs OVX control) was comparable to that of the sham group (\(P > 0.05\) vs sham). Neither the yam nor the genistein diet reversed the atrophy caused by OVX. The uterus weight in the yam and genistein groups was 22.0 ± 21.3 mg and 21.5 ± 9.5 mg, respectively (\(P > 0.05\) vs OVX control group).

Bone mineral density
The BMD of femur in the OVX group, 0.109 ± 0.011 g cm\(^{-2}\), was significantly lower than that in the sham group, 0.079 ± 0.015 g cm\(^{-2}\) (\(P < 0.05\) vs sham) (Fig. 1A). OVX mice with oestriadiol supplement had similar femoral BMD to sham-operated mice while mice in the yam or genistein group had greater BMD than the OVX control group (\(P < 0.05\) vs OVX control). Similarly, the BMD of lumbar bones were lower in the OVX group as compared with the sham group (\(P < 0.05\) vs sham). Mice fed the yam, oestriadiol or genistein diet had similar BMD to the sham-operated mice (\(P > 0.05\) vs sham).

Table 3. Body weight, feed intake and feed efficiency of sham-operated or ovariecotimised BALB/c mice fed control, yam, oestradiol or genistein diets for 12 weeks

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight gain (g)</th>
<th>Feed intake (g day(^{-1}))</th>
<th>Feed efficiency (10(^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated</td>
<td>6.7 ± 1.6(^{ab})</td>
<td>3.5 ± 0.3(^{bc})</td>
<td>2.2 ± 0.7</td>
</tr>
<tr>
<td>Control</td>
<td>6.7 ± 2.5(^{ab})</td>
<td>3.3 ± 0.2(^{ab})</td>
<td>2.5 ± 0.7</td>
</tr>
<tr>
<td>Yam</td>
<td>7.6 ± 1.7(^{a})</td>
<td>3.8 ± 0.2(^{a})</td>
<td>2.3 ± 0.5</td>
</tr>
<tr>
<td>Oestradiol</td>
<td>5.0 ± 0.8(^{a})</td>
<td>3.2 ± 0.2(^{a})</td>
<td>2.0 ± 0.5</td>
</tr>
<tr>
<td>Genistein</td>
<td>6.7 ± 2.5(^{ab})</td>
<td>3.3 ± 0.3(^{ab})</td>
<td>2.5 ± 0.6</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SD (n = 10–14). Different superscript letters denote significant differences between groups as analysed by one-way ANOVA followed by Duncan’s multiple test. Feed efficiency = daily body weight/ daily feed intake. No significant difference in feed efficiency was found between groups as analysed by Duncan’s multiple range test.
Ash content of femur and lumbar bones
The femoral ash content (mg) of OVX group was ∼15% lower than that in the sham group (P < 0.05 vs sham). The bone ash in the yam and genistein groups was not different to that in the sham group (P > 0.05 vs sham) (Fig. 1B). The ash contents of lumbar bones were significantly lowered by ∼26% in the OVX control group as compared to the sham group (P < 0.05 vs sham). This lumbar ash content was greater in the oestradiol and genistein groups as compared to the control group, respectively (P < 0.05 vs control).

Calcium content of femur and lumbar bones
There was a significantly lower femoral calcium content in OVX mice as compared to the sham-operated mice (P < 0.05 vs sham) (Fig. 1C). The femoral calcium content in mice fed yam and genistein diets was ∼73% (P < 0.05 vs OVX control) and ∼67% (P < 0.05 vs OVX control) of that in the sham group, respectively, while the oestradiol supplement fully preserved the bone calcium content (P > 0.05 vs sham). In terms of lumbar bones, the calcium content in the OVX, yam and genistein group was ∼61% (P < 0.05 vs sham), ∼81% (P > 0.05 vs sham) and ∼78% (P > 0.05 vs sham) of that in the sham-operated mice, respectively, while the E2 supplement fully preserve the calcium content (P > 0.05 vs sham).

Femoral cross-sections
The stained femoral sections are shown in Fig. 2. The thickness of cortical bone was 0.24 ± 0.05, 0.25 ± 0.05, 0.21 ± 0.05, 0.35 ± 0.10, and 0.28 ± 0.10 mm for the sham, control, yam, oestradiol, and genistein group, respectively. The trabecular bone fraction was 0.75 ± 0.05, 0.39 ± 0.07, 0.66 ± 0.05, 0.82 ± 0.05, and 0.61 ± 0.04 for sham, control, yam, oestradiol, and genistein group, respectively. The trabecular bone fraction of mice fed with yam and genistein diet was almost 88% and 81% of that in the sham group, respectively.

Plasma calcium
The plasma calcium concentrations were ∼2.0 mmol L⁻¹ for all, except OVX, groups of mice (Fig. 3). The plasma calcium level of the OVX group was ∼92% of that in the sham group (P < 0.05 vs all of the remaining four groups).

Short chain fatty acids in the caecum
The yam diet significantly increased acetic acid, propionic acid, butyric acid and total fatty acid concentrations in the caecal...
contents as compared to the other diets \( (P < 0.05 \text{ vs all of the remaining four groups}) \) (Fig. 4A). When the mass of caecal content was taken into account, the yam group still produced the greatest amount of acetic acid, propionic acid, butyric acid and total fatty acid \( (P < 0.05 \text{ vs all of the remaining four groups}) \) (Fig. 4B).

**Correlation between caecal total SCFA content and bone calcium content, or plasma calcium level**

The correlation coefficient between caecal SCFA content (mmol) and femur calcium content (mg), lumbar calcium content (mg) and plasma calcium level (mmol L\(^{-1}\)) was 0.07 \( (P > 0.05) \), 0.04 \( (P > 0.05) \), and 0.29 \( (P > 0.05) \), respectively.

**DISCUSSION**

Poulsen and Kruger\(^\text{11}\) recently summarised the phytoestrogen intervention animal studies and showed that most (90%) studies used rats, and only few studies used mice as the model. The O VX BALB/c mice model was first established in this study and we found it mimicked changes in bone metabolism observed in OVX rat\(^\text{22}\) as well as in human postmenopausal osteoporosis; that is, the BMD of OVX mice was apparently lowered compared to that of the sham-operated mice. This mouse model may offer another option to demonstrate the activity of phytoestrogenic compounds.

The present study showed that TNG yam and genistein diets prevented OVX-induced bone loss, but they did not exert oestrogenic activity on preventing the uterine atrophy. This organ-specific action of TNG yam on postmenopausal syndromes implies that TNG yam may act similarly to genistein as a selective oestrogen receptor modulator (SERM).\(^\text{8}\) This SERM role of TNG yam was supported by the study by Cheng et al.\(^\text{7}\) which demonstrates three out of five TNG yam phytoestrogenic compounds (\(\gamma\)-tocopherol-9, coenzyme Q9 and 1-feruloylglycol) are stronger agonists for oestrogen receptor-\(\beta\), while the other two compounds (\(\alpha\)-tocopherol and hydro-Q9 chromone) exert similar activity for oestrogen receptor-\(\alpha\) and oestrogen receptor-\(\beta\), based on the ligand-dependent transactivation assay of oestrogen receptors. We speculate that the phytoestrogens of TNG yam might act similarly to soy isoflavones that have been shown to inhibit parathyroid hormone-induced bone resorption\(^\text{9}\) and osteoclast activity.\(^\text{10}\)

Although the yam diet had a strong protective effect on BMD in either the femur or lumbar bone, it did not seem to fully protect the loss of ash content. The bone density (g cm\(^{-2}\)) measured by dual-energy X-ray absorptiometry reflects the overall density of all bone tissues, including both organic and inorganic matter, while ash content was composed of only inorganic matter. Therefore, our study suggested that the TNG yam diet maintained both the inorganic matter and collagen content of the bone.

The role of dietary protein intake on bone mass is not conclusive. Acidosis caused by high-protein intake is considered potentially detrimental for bone health in humans\(^\text{23,24}\), while other reports have emphasised that high protein intake benefits bone health.\(^\text{26–28}\) Since the protein content of TNG yam is greater than that of corn starch, we previously tested different protein levels of TNG diet in a pilot study. We found that when mice were fed an isoprotein yam diet in which casein was partially replaced by yam protein in order to provide equal amount of total protein as the control diet, the growth of mice was hampered as compared to the control group. However, this adverse effect was not observed when mice were fed a high-protein yam diet in which casein was maintained at the same level as the control diet in addition to the protein derived from yam. This observation was not surprising as Chen et al.\(^\text{29}\) have reported that diets consisting of greater than 250 g kg\(^{-1}\) raw TNG yam depressed the apparent absorption of dietary protein and lipid. Although the yam used in this study was cooked, it might still reduce the bioavailability of protein to some degree. Based on the role of protein on the bone structure\(^\text{26–28}\) and the growth effect of casein found in the pilot study, we decided to formulate a high-protein TNG diet for this study. This high-protein TNG diet was proven not to be a confounding factor.
in the bone protection effect of the yam diet, but may be beneficial in maintaining organic matter in bones.

The calcium intake and bioavailability of calcium could affect the bone mass. Therefore, it is essential to ensure similar calcium intake among different groups of mice. Although complete replacement of corn starch with TNG yam led to a slightly lower calcium content than in the control diet, daily calcium intake was not significantly different among groups when feed intake was taken into account. However, the bioavailability of dietary calcium was not examined in this study. Although we originally proposed that the TNG diet might increase the absorption of dietary calcium through fermentation products that increase the solubility of calcium, no significant correlation was found between caecal SCFA content and bone or plasma calcium levels. Since homeostasis of the plasma calcium level is tightly regulated, the potential increase in calcium absorption by TNG fermentation may not result in an increased plasma calcium level as the dietary calcium in the diet was sufficient (≥ 5 g kg⁻¹). Therefore, TNG yam may not prevent O VX-induced bone loss via modulation of calcium bioavailability, but mainly by direct phytoestrogenic effects.

CONCLUSION

The present study showed that, in O VX BALB/c mice, the TNG yam diet preserved BMD of femur and lumbar bone and plasma calcium concentration, but was completely ineffective in maintaining uterine mass. This bone-protective effect was likely to be mediated by phytoestrogenic compounds.

ACKNOWLEDGEMENTS

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